

SCREENING OF ANTIMICROBIAL RESISTANT BACTERIA IN WASTEWATER, VEGETABLES AND HUMANS

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Wastewater is used for irrigation in whole world now due to shortage of fresh water, people living in urban and pre-urban areas of developing countries, mostly utilize wastewater for irrigation. Faisalabad is an industrial city and large agricultural area in Faisalabad is irrigated with wastewater, which poses a potentially dangerous problem for the health of fresh vegetables and human. In Pakistan, demand of raw vegetables, fruits and sprouts is on the rise. Sale of prepared salad vegetables is very common in Pakistan. Spread of human diseases due to the pathogenic microbes on vegetables is usually caused by many factors, one of them is the fecal contamination because of manure fertilizers and contaminated wastewater used for irrigation. Wastewater and wastewater irrigated vegetables showed high no. of total microbial count (175×10^9 cfu/ml in wastewater), (159×10^8 cfu/ml in wastewater irrigated vegetables) and high no of coliform count are more dangerous. This study may help in promulgation and implementation of preventive and regulatory policies.

Keywords: wastewater, disc diffusion, total plate count, coliform count, Faisalabad

INTRODUCTION

Wastewater is used in agriculture all around the world, although the practice is variable in extent (Hamilton *et al.*, 2006). The declining fresh water for human use industries and ecosystem stability is now a universal problem. This problem need more effort than merely conserving the freshwater resources (Durán-Álvarez and Jiménez-Cisneros, 2014).

Around 1.2 million masses in developing countries are living in water scarce areas and the number is feared to be increased to about 1.8 million by 2025. The increased shortage of water in the arid areas have resulted in the application of wastewater in agriculture to mitigate the burden on freshwater resources. According to an estimate about 70% of treated wastewater is being used for agriculture, which may cause sinister environmental and health effects (Balkhair, 2016). Because of dry climate and growing urbanization, many farmers from developing countries having land in cities and rural regions now feed their crops and vegetables with wastewater. Use of wastewater is a serious health concern for the farmers, traders and handlers of these crops and the public that consume such vegetables (Alemayehu *et al.*, 2015).

Intestinal nematode, pathogenic bacteria (*Salmonella*, *Shigella*, *Escherichia coli* etc.) found in house hold sewerage water and animal sources are likely to contaminate the vegetables irrigated with this sewerage water (Balkhair, 2016; Alemayehu *et al.*, 2015). Above mentioned resistant microbes show resistant to extreme temperatures, desiccation, natural irradiation and commonly used disinfectants coupled with poor sanitation and inadequate personal hygiene contribute to

the contamination of vegetables as a result diseases may occur in consumers (Alemayehu *et al.*, 2015).

In Pakistan, demand of raw vegetables, fruits and sprouts is on the rise. Sale of prepared salad vegetables is very common in Pakistan. Spread of human diseases due to the pathogenic microbes on vegetables is usually caused by many factors some of them are the fecal contamination because of manure fertilizers and contaminated wastewater used for irrigation (Razzaq *et al.*, 2014). Application of wastewater for irrigation is considered harmful because of many reasons like it may produce odor, aversion of crops, addition of pollutants in the environment, increase the resistance of bacteria inhabiting the soil and outbreaks of diseases (Durán-Álvarez and Jiménez-Cisneros, 2014; Araviadis, 2015).

The unrefined wastewater and sewage water coming from textile industries contains number of chemicals including heavy metals, is applied in agricultural fields for raising vegetables and other plants. Wastewater containing loads of organic compounds leads to the proliferation of unwanted microorganisms (Debnath *et al.*, 2014).

Human health is at risk of bacteria that are antibiotic resistant but some knowledge is present about the spreading of ESBL generated by *Escherichia coli* from the wastewater, hospital and their nearby vicinity. Detection of bacteria from hospitals and untreated sewage water is very attention-grabbing, and their level of release to the environment (Korzeniewska *et al.*, 2013).

The human pathogens form few decade of history getting antibiotic resistance which is alarming situation for the health of entire world. Genes named as resistome are discovered

from metagenomic as well as genomic studies in humans, animals, foods and their atmosphere (Rolain, 2013).

A lot of antimicrobial agents are being used in animal food production that is the main cause of antimicrobial resistance around the world. Subsequently the check and balance of the excessive use of antimicrobial agents should be there to low down the resistance of microbes (Garcia-Migura *et al.*, 2014). The present study was planned the check the pathogenic microorganism that are resistant to antibiotics, the cyclic movement of resistant pathogenic microbes from humans to wastewater and to vegetables etc. and to check the microbial load on wastewater irrigated vegetables and in wastewater.

MATERIALS AND METHODS

Study area: Wastewater and vegetable samples irrigated with wastewater were collected from different areas of district Faisalabad. Research were performed in the Institute of Microbiology, University of Agriculture Faisalabad. In different areas of Faisalabad like Samanabad, Sitara Colony, Ghulshan Iqbal, Chak Risalewala, Sadhar and near new campus of GC University Faisalabad etc. untreated sewage is irrigated regularly to the vegetables. Most polluted site receiving the water from the sewage pumps and discharge of various industrial effluents.

Sample Size : Fifty samples for each variable (Vegetables and Wastewater) and forty samples were collected for human stool.

Sample collection: Wastewater samples were collected in 250 ml sterile bottles from different sewage outlets from district Faisalabad. The collection points were close to the pumps used for irrigation. Vegetables crops available at the time of visit were purchased from the local villagers, cut into pieces using alcohol sterilized scissors and packed in polyethylene bags. All samples were transported in cold packs to the laboratory and were analyzed within 7 h of collection.

Human sample collection: Stool samples were collected from humans in a 50 ml sterile plastic container, these samples were further processed for inoculation.

Enumeration, isolation and characterization of isolates: Twenty-five grams of each vegetable were washed by shaking thoroughly with 25 ml of 0.1% sterile peptone water. Appropriate dilutions of the irrigation water and vegetable washings were inoculated in duplicates by spread plating on MacConkey Agar and plate count agar for enumeration of total coliforms and viable counts, respectively. Typical lactose fermenting colonies on MacConkey Agar and all cells on plate count agar were counted after incubation at 37°C for 24 h. Vegetable washing (25 ml) were pre-enriched in 225 ml sterile peptone water. One milliliter of the pre-enrichment were sub-cultured for selective enrichment.

Antibiotics sensitivity test : Mueller Hinton Agar (MHA) were used for antibiotic sensitivity test.

Antibiotic sensitivity were checked against the following antibiotics in bacteria as under:

Amikacin, Ciprofloxacin, Erythromycin, Cefotaxime, Chloramphenicol, Trimethoprim and Tigecycline.

RESULTS AND DISCUSSION

Wastewater is used for irrigation all over the world, this practice is mostly observed in developing countries, because of the lack of fresh irrigation water. Faisalabad is an industrial city and large agricultural area in Faisalabad is irrigated with wastewater, which poses a potentially dangerous problem for the human health.

Vegetable is a plant or part of a plant used as food, such as a Spanish, lettuce, cabbage, potato, turnip etc., most of the vegetables are perishable and microorganisms present in wastewater put deleterious effect on these vegetables. Both the vegetable (Lettuce, Spanish, coriander) and wastewater were analyzed for TPC and TC count, there were a very high count for both. TPC count for vegetables was 1.62×10^{10} cfu/g and for wastewater was 1.75×10^{11} cfu/ml. TC count was 6.1×10^6 cfu/g and 6.5×10^7 cfu/ml, respectively.

The counts were clearly above the acclaimed standards for ready to eat vegetables specifically coliforms which should be less than 10 /g. The higher the number of microorganisms present on a food item there are more chance of disease befalling. The Wastewater is known to cause many human disease outbreaks in various parts of the world. Most of the studies discourage the use of wastewater for vegetables production. Human enteric pathogenic bacteria (*Salmonella*, *Shigella*, *Escherichia coli* etc.) found in wastewater were reported to contaminate the vegetables irrigated with this wastewater.

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The counts were clearly above the acclaimed standards for ready to eat vegetables specifically coliforms which should be less than 10 /g. The higher the number of microorganisms present on a food item there are more chance of disease befalling (Table 1). The Wastewater is known to cause many human disease outbreaks in various parts of the world. Most

of the studies discourage the use of wastewater for vegetables production (Table 2). Human enteric pathogenic bacteria (*Salmonella*, *Shigella*, *Escherichia coli* etc.) found in wastewater were reported to contaminate the vegetables irrigated with this wastewater.

Table 1: Average no. TPC / TC Count (cfu/ml) in Wastewater of District Faisalabad

Serial no	Location in district Faisalabad	Total plate count /ml	Total coliform count /ml
1	Sitara Colony	175x10 ⁹	57x10 ⁶
2	Samanabad	170x10 ⁹	61x10 ⁶
3	Nawabanwala	145x10 ⁹	64x10 ⁶
4	Ghulshan e Iqbal park	146x10 ⁹	60x10 ⁶
5	Sadhar	160x10 ⁹	44x10 ⁶
6	Chak no 132 JB	143x10 ⁹	65x10 ⁶

Table 2: Average no. TPC / TC Count (cfu/g) in Wastewater Irrigated Vegetables of District Faisalabad

Serial no	Location in district Faisalabad	Total plate count /ml	Total coliform count /ml
1	Sitara Colony	159x10 ⁸	57x10 ⁵
2	Samanabad	139x10 ⁸	61x10 ⁵
3	Nawabanwala	150x10 ⁸	64x10 ⁵
4	Ghulshan e Iqbal park	162x10 ⁸	60x10 ⁵
5	Sadhar	126x10 ⁸	44x10 ⁵
6	Chak no 132 JB	132x10 ⁸	65x10 ⁵

Escherichia coli and Salmonella. Antibiotics susceptibility was performed on Muller Hinton agar by Kirby Bueyer method and evaluated the zone of inhibition. (Singh *et al.*, 2011). Wastewater isolates shows percentage resistance against the following antibiotics Amikacin (AK) 40%, Ciprofloxacin (CIP) 00%, Erythromycin (E) 50%, Cefotaxime (CTX) 44%, Chloramphenicol (C) 00%, Trimethoprim (W) 52%, and Tigecycline (TGC) 40%. Similar pattern is shown by the wastewater irrigated vegetable isolates, Amikacin (AK) 38%, Ciprofloxacin (CIP) 00%, Erythromycin (E) 34%, Cefotaxime (CTX) 46%, Chloramphenicol (C) 00%, Trimethoprim (W) 66%, and Tigecycline (TGC) 34%. Human stool isolates were also resistant towards antibiotics, Amikacin (AK) 50%, Ciprofloxacin (CIP) 05%, Erythromycin (E) 65%, Cefotaxime (CTX) 48%, Chloramphenicol (C) 00%, Trimethoprim (W) 60%, and Tigecycline (TGC) 45%. In case of Human stool isolates there are three antibiotics that are

resistant above 50%, that is Amikacin 50%, Erythromycin 65% and Trimethoprim is 60% Figure 1.

Some of the bacteria isolated in this study may be part of the natural flora of the fruits and vegetables or contaminants from soil, irrigation water, and the environment during transportation, washing/rinsing water or handling by processors (Ofor *et al.*, 2009).

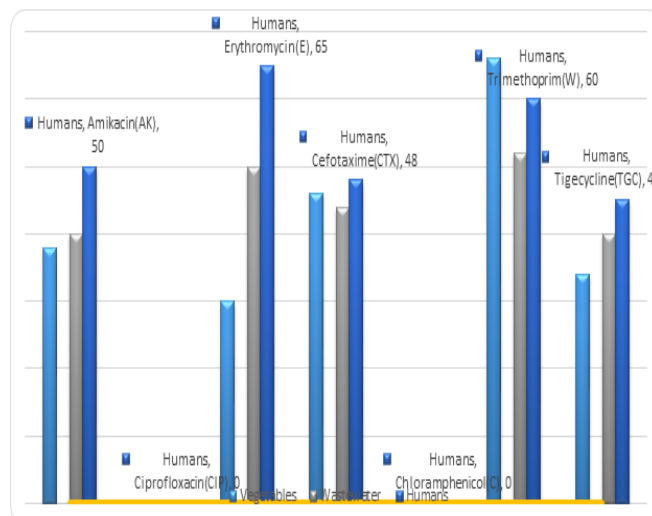


Figure 1: Comparison of Antimicrobial Resistance in Wastewater, Vegetables and Humans

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